

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:

Source: [Cornell Sequencing Handbook](#)

Primary Experimenter (contact):
Other Experimenters:

Assembled: 6/27/2012

Reagent	Details	Quantity
ddH2O (nuclease-free)		Up to 18 <input type="checkbox"/> L
Primer (10 <input type="checkbox"/>M_stock)	Conc. labeled on microfuge tubes in primer box	1 <input type="checkbox"/> L
DNA template		*Var.
Sequencing tube		

***Plasmid**, ~1 ☐g; **PCR product**, #base pairs/5.0 = amount of PCR product in ng that we need
 Example: 250bp PCR product. 250bp ÷ 5.0 = 50ng of DNA + 8 pmole primer in 18ul (Note: maximum PCR product concentration is 100ng/ul)

Order #	Template	Template conc. (ng/ <input type="checkbox"/> L)	Template volume (<input type="checkbox"/> L)	Primer ID
-	Plasmid ID , Sample name; Ex) p8, Gibson 1			Ex) 0033
1				
2				
3				
4				
5				

6				
7				
8				
9				
10				
11				
12				
13				
14				
15				

NOTE: Please write calculations on back of sheet.

Procedure:

Critical Steps:

- Clearly label all sequencing tubes before beginning
- NEVER add primers directly from blue cap tubes, only the diluted 10 μ M stocks

NOTE:

For primers that aren't strictly sequencing primers, stocks are 10 μ M. These can still be used for sequencing.

c Label microcentrifuge tubes

- o Write the order # on the top of the tube
- o Write the Order name on the side of the tube
- o Ex) "Gibson 1" on side, "1" on top

c Calculate μ L of DNA template to add to reach desired amount

$$\frac{\text{Desired ng DNA}}{X \text{ DNA template } \left(\frac{\text{ng}}{\mu\text{L}} \right)} = (Y \mu\text{L to add for desired ng DNA})$$

- o Aim for \sim 1 μ g for plasmid DNA
- o Please write calculations on backs of the front pages of this protocol

c Calculate μ L of ddH₂O to add

- o (17 μ L) – (Y μ L to add for desired μ g DNA) = (μ L H₂O to add)
- o Please write calculations on backs of the front pages of this protocol

c Add ddH₂O, primer, and finally DNA template to tube

- o Add primer from the 10 μ M stock

c Place all tubes into a Ziploc bag and record the date & order ID from BRC on bag

